A non-major histocompatibility locus determines tissue specificity in the pathogenic process underlying synovial proliferation in a mouse arthropathy model.

<table>
<thead>
<tr>
<th>著者</th>
<th>Zhang Ming-Cai, Mori Shiro, Date Fumiko, Furukawa Hiroshi, Ono Masao</th>
</tr>
</thead>
<tbody>
<tr>
<td>会議名</td>
<td>Annals of the Rheumatic Diseases</td>
</tr>
<tr>
<td>卷</td>
<td>66</td>
</tr>
<tr>
<td>号</td>
<td>2</td>
</tr>
<tr>
<td>頁</td>
<td>242-245</td>
</tr>
<tr>
<td>年</td>
<td>2007</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10097/51627">http://hdl.handle.net/10097/51627</a></td>
</tr>
<tr>
<td>doi</td>
<td>10.1136/ard.2006.054999</td>
</tr>
</tbody>
</table>
A non-major histocompatibility locus determines tissue specificity in the pathogenic process underlying synovial proliferation in a mouse arthropathy model

Ming-Cai Zhang, Shiro Mori, Fumiko Date, Hiroshi Furukawa, Masao Ono

Background: The incidence and characteristics of spontaneous ankylosis in the ankle of specific F1 mice descended from two Faslpr-deficient strains were reported. Here the coincidence of synovial proliferation and ankylosis in the descendant F2 mice is reported.

Aim: To clarify whether the two distinct manifestations are genetically different.

Methods: An arthropathic group of mice (MCF2) were bred by intercrossing MRL/Mp,Faslpr⁻/− and C3H/He,Faslpr⁻/− mice. All mice were killed by bleeding under anaesthesia when they were 6 months old. Pathological grades for synovial proliferation were determined by microscopical examination. To obtain a linkage locus, the whole genome of male MCF2 mice was scanned by using 73 microsatellite markers.

Results: Synovial proliferation was equally observed in male and female MCF2 mice. No correlation was observed between the grades of synovial proliferation and the ankylosis occurring in the MCF2 mice. A suggestive susceptibility locus was shown in the middle of chromosome 11. This locus was an MRL allele with a recessive inheritance mode.

Conclusion: The pathogenic mechanisms of synovial proliferation and ankylosis are genetically different. The present locus is overlapped with some loci associated with rheumatoid arthritis and with others associated with experimental arthritides.

A n inflammatory joint disease is usually recognised as an admixture of destruction and proliferation of the joint components. An imbalance of such counterprocesses may result in a pathological remodelling of joint structures giving rise to a unique clinicopathological outcome of the disease. Rheumatoid arthritis is characterised by synovial proliferation and erosion of joint cartilage due to chronic inflammation. The remodelling process in rheumatoid arthritis is associated with the contraction and destruction of joints. On the other hand, an ankylosing disease, another type of inflammatory joint disease, involves a distinct remodelling process causing joint ankylosis. Although the remodelling process for ankylosis has not been completely characterised, recent studies using rodent ankylosis models have evaluated the pathological proliferation of a particular region, where a joint capsule or ligament attaches to a bone.1-4 The histological characteristics are proliferation, cartilage formation and, subsequently, replacement of cartilage by bone, a process typical of endochondral bone formation. The process is called ankylosing enthesitis. A comparative study on the distinct joint remodelling processes may shed new light on the distinct and essential mechanisms of rheumatoid arthritis and ankylosing diseases.

We recently reported a mouse model with spontaneous and male-predominant onset of progressive ankylosis in ankle joints.5 It should be noted that this joint ankylosis occurred in a particular F1 generation of mice, which descended from two ankylosis-free strains—MRL/Mp,Faslpr⁻/−,sap⁻/⁻ (previously denoted as MRL/rpl)6 and C3H/He,Faslpr⁻/− (C3H/lpr). The MRL/rpl strain spontaneously arose from a lupus-prone MRL/Mp,Faslpr⁻/− (MRL/lpr) strain. It is noteworthy that the mutation on signalling lymphocyte activation molecule (SLAM)-associated protein (SAP) attenuated the development of autoimmune diseases as seen in MRL/Mp,Faslpr⁻/− (MRL/lpr) mice.6 These mice develop spontaneous arthritis characterised by synovial proliferation; therefore, they are considered as a model for rheumatoid arthritis. The onset of ankylosis in (MRL/rpl×C3H/lpr) F1 mice was unexpected. A defect of SAP or a coupling of two predisposed genes descended from the two parental strains was a possible interpretation for this onset. To deal with this issue, we performed a linkage analysis using (MRL/rpl×C3H/lpr) F2 (MCF2) mice and arrived at two genetic mechanisms for the onset of ankylosis in these mice: the dissociation of the SAP defect and association of the single locus on chromosome 7 with the onset of ankylosis.7 Although the question is not completely answered, the results provided an opportunity to characterise a non-major histocompatibility complex (MHC)-linked locus associated with the pathogenesis of ankylosis.

In this study, we observed sporadic incidences of synovial proliferation and ankylosis (enthesal proliferation) in MCF2 mice. The relationship between the pathogenic mechanisms of synovial and enthesal proliferations has been discussed. We provide evidence that the two distinct joint manifestations independently develop and are controlled differently by distinct genetic loci.

METHODS

Mice

C3H/lpr mice were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA) and bred under specific pathogen-free conditions in the Animal Research Institute, Tohoku University Graduate School of Medicine, Sendai, Japan. MRL/rpl, MCF2, and MCF3 mice were housed in the same condition. In all animal experiments, we observed the Tohoku University guidelines for animal experimentation.

Histopathological grading of ankylosis

All mice were killed at 6-months of age under ether anaesthesia. Ankle joints were fixed in 10% formalin buffered with 0.01 M phosphate (pH 7.2), decalcified in 5% formic acid and 5% formalin and embedded in paraffin wax. Tissue sections were stained with haematoxylin and eosin. The pathological grade of the synovial proliferation was determined according to the following criteria: normal (grade 0), multiplication of synovial lining layer (grade 1) and grade 1 with villous

Abbreviations: MCF2, (MRL/rpl×C3H/lpr)F2; SAP, signalling lymphocyte activation molecule-associated protein; SLAM, signalling lymphocyte activation molecule
proliferation (grade 2). The grade of an individual mouse was defined as a maximal grade determined in six longitudinal sections of bilateral hind paws. Samples inappropriate for microscopical examination, mainly because of the absence of synovium in accountable sections, were precluded from further analysis. The number of female and male MCF2 mice accounted in this study was 111 and 81, respectively. The grade of ankylosis was determined previously.5

Genomewide screening for susceptibility loci to synovial proliferation

We searched association loci in the whole genome using 81 male MCF2 mice and 73 microsatellite markers, which were all able to distinguish the parental genotypes and provided a coverage of the mouse genome with an average distance of 23.3 cM apart. The genetic positions of microsatellite markers and genes were determined on the basis of the Mouse Genome Informatics provided by The Jackson Laboratory.

Statistical analysis

A correlation between the grades of synovial proliferation and ankylosis was evaluated by Fisher’s exact test with standard 3 × 4 contingency matrices. An association of the onset of synovial proliferation and the genotype at each marker position was evaluated by the χ² test with standard 2 × 3 contingency matrices. A significant or suggestive association was estimated by p < 0.000052 or 0.0016, respectively, as recommended by Lander and Kruglyak.7

Table 1

<table>
<thead>
<tr>
<th>Marker</th>
<th>Position</th>
<th>Incidence of synovitis</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MM</td>
<td>MC</td>
<td>CC</td>
</tr>
<tr>
<td>D11Mit71</td>
<td>1.1</td>
<td>13</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>D11Mit263</td>
<td>55.6</td>
<td>9</td>
<td>32</td>
<td>11</td>
</tr>
<tr>
<td>D11Mit334</td>
<td>68</td>
<td>11</td>
<td>29</td>
<td>12</td>
</tr>
<tr>
<td>D11Mit338</td>
<td>75</td>
<td>10</td>
<td>30</td>
<td>12</td>
</tr>
</tbody>
</table>

Genotypes MM, MC and CC indicate MRL/MRL homozygote, MRL/C3H heterozygote and C3H/C3H homozygote, respectively.
RESULTS
Incidence of synovial proliferation
In addition to the onset of ankylosis, we observed a thickening of the synovial lining layer, with mild lymphocytic infiltration and proliferation of small vessels in MCF₂ mice (fig 1A,B). Villous formations were evident in the advanced stage of the synovial proliferation. Neither lymphoid aggregation in the synovial lesion nor bone erosion with pannus-like formation was detected in all the mice examined. The microscopical incidence of synovial proliferation was 12/25 (48%) in MRL/lpr, 12/26 (46.2%) in C3H/lpr, 14/69 (20.3%) in MCF₁ and 69/192 (35.9%) in MCF₂ mice. The synovial lesion was detected equally in male and female MCF₂ mice (p = 0.973) (fig 1C). No significant correlation was obtained between the progression of ankylosis and synovial proliferation in male-only (p = 0.258, fig 1D) and all MCF₂ mice (data not shown).

Mapping of susceptibility loci associated with synovial proliferation
In this mapping study, we analysed the same MCF₂ mice that were used in the previous mapping study on ankylosis. The genomewide search using 81 male mice with a single marker D11Mit263 (55.6 CM) on chromosome 11 that was significantly associated with the onset of synovial proliferation (p<0.001; table 1). The incidence of synovial proliferation in the MRL homozygote group (17/26, 65.4%) in association with D11Mit263 was higher than that in the MRL/C3H heterozygote (8/40, 20%) and C3H homozygote (4/15, 26.7%) groups. This indicates that this MRL-derived locus regulates the onset of synovial proliferation in a recessive inheritance manner. No significant association was observed between synovial proliferation and any marker on the X chromosome where the SAP gene exists.

DISCUSSION
Recent studies have reported several mouse models with spontaneous ankylosis. DBA/1 is a spontaneous ankylosis model and an induced synovitis model with immunisation of type II collagen. The two DBA/1-related arthropathies develop similarly in a male-predominant fashion, although they are histopathologically different. It has been of particular interest to determine whether the pathogenic mechanisms of the distinct arthropathies in the DBA/1 mice are overlapped.

A cross mating of the DBA/1 strain with a particular lupus-prone strain such as BXSB or MRL/Mp resulted in an acceleration of the onset and increase in the severity of ankylosis in F₁ mice.¹ ² These facts may suggest a correlation between lupus susceptibility and the pathogenesis of ankylosis. The MCF₂ mice used in this study also shared lupus-prone and synovitis-prone genetic backgrounds with MRL/lpr mice to a lesser extent than those in the original MRL/lpr mice. We thought that this was a counterfact of ankylosis on synovitis; however, this notion turned out to be erroneous. This may be partly due to the difference in bleeding condition and genetic effects derived from the C3H/lpr strain. Our results totally revised these considerations and enforced a notion that proliferating enthesis entail specific mechanisms for the development of ankylosis.

Our results suggest a susceptibility locus in the middle of chromosome 11. As the number of markers and samples were not sufficient to detect complex loci, some loci may have remained undetected. However, the detected locus has a major effect on the development of synovial proliferation. This locus contains several genes that are highly conserved in a particular interval of human chromosome 17. To date, a high-resolution linkage and association mapping study identified a susceptibility locus for rheumatoid arthritis in this region.³ ⁴ The central part of mouse chromosome 11 also contains several loci associated with experimental autoimmune encephalomyelitis,⁵ ⁶ collagen-induced arthritis⁷ ⁸ and proteoglycan-induced arthritis.⁹ ¹⁰ This region is noteworthy when considering the genetic predisposition of rheumatoid arthritis.

A previous study using a larger number of backcross mice prepared from the same parental strains of MRL/Mp and C3H/He indicated that the five linkage loci are associated with the onset of arthritis.¹¹ None of these loci overlap with the present linkage region. This discrepancy is possibly due to a difference in the mouse generation used (N₂ or F₂), a difference in the housing condition or a suppressive effect of the Y chromosome on the onset of arthritis.¹² None of these loci overlap with the present linkage region. This discrepancy is possibly due to a difference in the mouse generation used (N₂ or F₂), a difference in the housing condition or a suppressive effect of the Y chromosome on the onset of arthritis.¹²

ACKNOWLEDGEMENTS
We thank Ms N Yamaki, Dr N Tanda and Dr H Umeda for technical assistance, Mrs N Fujisawa for secretarial assistance and Dr M Nose for critical comments.

Authors’ affiliations
Ming-Cai Zhang*, Fumiko Date, Hiroshi Furukawa, Masao Ono, Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan
Shiro Mori*, Department of Oral Medicine and Surgery, Tohoku University Graduate School of Dentistry, Sendai, Miyagi, Japan
*These authors contributed equally to this work.

Funding: This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan to SM (number 15390607) and MO (number 16390113).

Competing interests: None.

Correspondence to: Dr Masao Ono, Department of Pathology, Tohoku University Graduate School of Medicine, 2-1 Seiryo, Aoba, Sendai, Miyagi 980-8575, Japan; onomasao@mail.tains.tohoku.ac.jp

Accepted 10 July 2006

Published Online First 25 July 2006

REFERENCES
A synovitis-specific locus in an arthropathic strain MRL/lpr

and spinal cord lesions in experimental allergic encephalomyelitis. Am J Pathol
13 Liljander M, Sallstrom MA, Andersson S, Andersson A, Holmdahl R, Mattsson R.
Identification of collagen-induced arthritis loci in aged multiparous female mice.
Arthritis Res Ther 2006;8:R45.
14 Adarichev VA, Nesterovitch AB, Bards T, Biesczat D, Chandrasekaran R,
Vermes C, et al. Sex effect on clinical and immunologic quantitative trait loci in a

quantitative trait loci controlling development of experimental autoimmune
encephalomyelitis and proportion of lymphocyte subpopulations. J Immunol

Butterfield RJ, Blankenhorn EP, Roper RJ, Zachary JF, Doerge RW, Teuscher C.
Identification of genetic loci controlling the characteristics and severity of brain

arthritis susceptibility locus homologous to one linked to two rat models of

quantitative trait loci controlling development of experimental autoimmune
encephalomyelitis and proportion of lymphocyte subpopulations. J Immunol

12 Butterfield RJ, Blankenhorn EP, Roper RJ, Zachary JF, Doerge RW, Teuscher C.
Identification of genetic loci controlling the characteristics and severity of brain

www.annrheumdis.com

Downloaded from ard.bmj.com on August 21, 2011 - Published by group.bmj.com
A non-major histocompatibility locus determines tissue specificity in the pathogenic process underlying synovial proliferation in a mouse arthropathy model

Ming-Cai Zhang, Shiro Mori, Fumiko Date, et al.

doi: 10.1136/ard.2006.054999

Updated information and services can be found at:
http://ard.bmj.com/content/66/2/242.full.html

These include:

References
This article cites 13 articles, 6 of which can be accessed free at:
http://ard.bmj.com/content/66/2/242.full.html#ref-list-1

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Genetics (1634 articles)
- Immunology (including allergy) (45835 articles)
- Connective tissue disease (7720 articles)
- Degenerative joint disease (9992 articles)
- Musculoskeletal syndromes (17637 articles)
- Rheumatoid arthritis (3811 articles)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/